

Study Title:

Request for Waivers of Environmental Fate Studies  
for Silver Dihydrogen Citrate Products

Data Requirements:

OCSPP 835.1110: Activated Sludge Sorption Isotherm  
OCSPP 850.6800/850.3300: Modified Activated Sludge Respiration Inhibition

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VP, Product Development/Regulatory Affairs

Date:

09.26.2020

## GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This volume provides the rationale for waiver requests. The following exposure and risk assessment information is not subject to the principles of the U.S. Environmental Protection Agency's Good Laboratory Practice (GLP) Standards as set forth in 40 CFR Part 160.

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## OVERVIEW OF REQUEST FOR STUDY WAIVERS

Waivers are requested from the requirements to conduct OCSPP 835.1110 and 850.6800 as requested by the July 6, 2012 Generic Data Call-In, GDCI-072500-1194 (2012 GDCI), and reinstituted in September 2019. Per the letter from EPA on September 3, 2019, all the ETI H<sub>2</sub>O silver dihydrogen citrate (SDC) products have been reclassified from Silver (elemental), PC Code 072501, to Silver ion, PC Code 072500.<sup>1</sup>

### **Introduction & Background**

In the 2019 letter, EPA has reinstituted its previous request that that two environmental fate studies be submitted on our silver-containing antimicrobial products. Per the 2012 GDCI and 2019 letter, the Agency has requested the following studies:

OCSPP 835.1110: Activated Sludge Sorption Isotherm

OCSPP 850.6800: Modified Activated Sludge Respiration Inhibition

The activated sludge sorption isotherm (ASSI) data guideline (i.e. 835.1110<sup>2</sup>) states “information on sorption potential is needed to assess the possibility for the removal of chemical compounds in biological wastewater treatment systems” and outlines a procedure for the “determination of the sorption potential of activated sludge solids for removal of specific chemical compounds.” Specifically, a “sorption isotherm” is developed for “measuring the extent to which a chemical compound distributes itself between activated sludge as the sorbent and water as the solvent.”

The activated sludge respiration inhibition (ASRI) test (i.e. 850.6800<sup>3</sup>) is intended to “provide a rapid screening method whereby test substances which may adversely affect aerobic microbial treatment plants can be identified and to indicate suitable non-inhibitory concentrations of test substances to be used in biodegradability tests.” The goal is to “determine the quantity of test substance required to cause a 50 percent inhibition or reduction (IC<sub>50</sub>) in respiration” at 3 hours. The normal microbial inoculum for performing this assay is activated sludge from a sewage treatment plant “treating predominantly domestic sewage” or from “sewage works treating predominantly industrial waste water.”

ETI H<sub>2</sub>O, a division of PURE Bioscience produces four (4) silver dihydrogen citrate containing products.<sup>4</sup> The MUP concentrate (Product 1 below) contains 2400 ppm or 0.240% silver ion and 20.66% citric acid with the remainder consisting of deionized water, produced in an integrated system. Silver *per se* is not present in an isolated form; it exists only as an ion stabilized by citric

<sup>1</sup> See letter from EPA to Steptoe & Johnson LLP, dated September 3, 2019. Subject: Amendment to the Generic Data Call-In Notices for Products Containing Silver Particles.

<sup>2</sup> The January 1998 version of 835.1110 is available at <https://www.regulations.gov/document?D=EPA-HQ-OPPT-2009-0152-0003>

<sup>3</sup> Guideline 850.6800 is available at <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0152-0003>. In January 2012, this guideline was adopted in final form as 850.3300 and is available at <https://www.regulations.gov/document?D=EPA-HQ-OPPT-2009-0154-0021>. All references to 850.6800 throughout the document also refer to 850.3300.

<sup>4</sup> See 74 FR 27745, July 10, 2009, where the Agency identifies this product as “Silver ions resulting from the use of electrolytically-generated silver ions stabilized in citric acid as silver dihydrogen citrate.”

acid. There is no particulate matter in these products, nor are there any nanoscale particles of silver.<sup>5</sup>

The four registered products are:

1. Axenohl (Reg. No. 72977-1) which is 2400 ppm or 0.240% silver ion and 20.66% citric acid. This is the manufacturing use product (MUP).
2. Axen30 (Reg. No. 72977-3) which is 30 ppm or 0.003% silver ion, 4.8% citric acid
3. Axen50 (Reg. No. 72977-4) which is 50 ppm or 0.005% silver ion, 5% citric acid.
4. SDC3A (Reg. No. 72977-5) which is 30 ppm or 0.003% silver ion, 4.8% citric acid.

The MUP is used solely for making antimicrobial end-use products and ready-to-use sprays and solutions for indoor use (Products 2 through 4 above). Application of the product is achieved via non-aerosol-generating-method (i.e. spray trigger, pump spray, etc.). The directions for use specify that no potable water rinse is needed following application and surfaces can either be wiped with a clean towel or allowed to air dry after sufficient contact time.<sup>6</sup>

### **Regulatory Basis For Requested Waivers**

At 40 CFR 158.45, the Agency identifies that waivers for data requirements can be considered:

- a) The data requirements specified in this part as applicable to a category of products will not always be appropriate for every product in that category. Some products may have unusual physical, chemical, or biological properties or atypical use patterns which would make particular data requirements inappropriate, either because it would not be possible to generate the required data or because the data would not be useful in the Agency's evaluation of the risks or benefits of the product. The Agency will waive data requirements it finds are inappropriate, but will ensure that sufficient data are available to make the determinations required by the applicable statutory standards.

Agency's strategic objective for toxicity testing, as identified at <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-21st-century-science>, clearly identifies the Agency's objective of "Refining and reducing animal testing by maximizing information obtained from animal studies, and focusing on effects of concern" and moving from testing for 'completeness' to carefully focused animal testing where concerns exist, using ..." hazard-based hypotheses about the plausible toxicological potential of a pesticide or group of pesticides based on their physical-chemical properties." This is consistent with the National Academies of Sciences recommendations to EPA and is an essential component of the Agency's objectives.<sup>7</sup>

<sup>5</sup> The original DCI requirements from 2012 were placed on hold in 2015 pending an EPA review intended to determine whether existing registered products should be reclassified as "nanosilver." See letter from EPA from September 3, 2019.

<sup>6</sup> For example, see Directions for Use for Axen 50 (EPA Registration # 72977-4) label dated August 6, 2009.

<sup>7</sup> The recommendations provided to EPA can be found in the 2007 National Academies of Science/National Research Council report, "Toxicity Testing in the 21st Century: A Vision and a Strategy." Information regarding the implementation of these recommendations can be found here: <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-21st-century-science>.

### **Rationale for waivers**

According to the antimicrobial pesticide data requirements at 40 CFR Part 158 Subpart W, the ASSI test (OCSPP 835.1110) is not required for compounds that are relatively volatile (but not hydrophobic), highly reactive, or if the log Kow is less than 3. As estimated by KOWWIN, Version 1.67, the log Kow for silver ion is 0.23, and citric acid has a Kow of -1.72 indicating very low potential for solubility in lipids as well as bioaccumulation (ChemSpider, 2012)<sup>8</sup>. SDC is comprised of ionic silver and citric acid and it is not expected that the Kow of SDC would be above either of the individual components as SDC is completely water soluble. Neither silver ion nor citric acid meets the basic triggers for requiring 835.1110, per Part 158W at 40 CFR §158.2280, notes 19 and 20.

The indoor use of SDC-based products is unlikely to result in appreciable amounts of free silver ion due to a lack of a mobility pathway into the environment, as well as ample opportunities for free silver ions to be bound and removed by waste water treatment plants (WWTPs), which display a high degree of removal of silver ion from influent waste water streams. The public literature contains sufficient data on the behavior of silver ion in natural aquatic (both freshwater and marine) and WWTP environments, including information sought by OCSPP 850.6800 and 835.1110. In general, silver binds strongly to sulfur (inorganic or organic) in natural aquatic systems (freshwater and marine), as well as WWTPs, and this sulfidation results in significantly decreased toxicity from the lower solubility of silver sulfide (Levard et al., 2012). Any additional data on SDC-based products would not contribute to the existing knowledge.

### **No viable mobility pathway exists for indoor use of SDC products**

ETI H2O's products consist of a MUP for making antimicrobial end-use products and the resulting ready-to-use sprays and solutions. All of the products are registered only for indoor use and do not have any outdoor use patterns. Application of the product is achieved via non-aerosol-generating-method (i.e. spray trigger, pump spray, etc.) followed by wiping or air drying. The label directions do not require the product be wiped up after application and there is no need for a potable water rinse following application. In fact, for certain use sites (such as meat, poultry and dairy processing plants), the use of a potable water rinse following application is not allowed (for example, see the Axen 50 label).

As such, there is no viable "mobility pathway" for residues on indoor surfaces to reach the outdoor environment, much less the non-target plants and organisms found there. Furthermore, any "down the drain" off-site transport of silver ion would be rapidly transformed to other less mobile and less toxic forms. This is discussed in more detail below.

### **Silver is mostly removed by WWTPs**

Any "minimal runoff" associated with indoor use of SDC products could occur in one of two ways: (1) "down the drain" runoff following off-label use (i.e. using a potable water rinse and disposing of rinsate in the sink); (2) or through land application of wastewater sludge biosolids for fertilization purposes. In the former case, the vast majority of free silver ion (as provided by

<sup>8</sup> ChemSpider, Accessed June 11, 2012. Available at: <http://www.chemspider.com/Chemical-Structure.94565.html>

release from SDC) will be removed during the wastewater treatment process, while in the latter case, the species of silver found in biosolids (silver sulfide) poses very low toxicity to plants due to the extremely strong nature of the covalent bond between the silver and sulfide ions.

In considering the effect of down-the-drain disposal of silver-bearing surficial residues, it is useful to consider a “life cycle” approach to follow the fate and transport of silver ion from its application as an SDC spray into the environment.

This can be split into three areas: (1) pre-treatment (from the home to a wastewater treatment plant); (2) treatment; and (3) post-treatment (transformation in the environment following treatment):

(1) Pre-Treatment – In traveling from home plumbing to the WWTP, there is ample opportunity for positively-charged silver cations to bind to negatively-charged anions, to sorb to organic matter in the water column, or to sorb onto some other reactive surface. Silver has the strongest binding preference for reduced sulfur species<sup>9</sup> such as sulfates (EPA, 2010), and sulfur in domestic wastewater is both readily available (from urine) and predominantly present as sulfate (Kaegi et al., 2011). Silver ion also bonds to the ample amount of chloride ions present in natural and waste waters that mix in the sewer pipe, forming insoluble silver chloride. These strong bonds result in immobilization in wastewater and surface waters (Adams and Kramer, 1999). In addition to binding to other ligands, silver ions are also attracted to negatively-charged fulvic and humic acids that are found in organic matter (EPA, 2010). Several of the studies cited below demonstrate that the transformation of silver ion into silver chloride and silver sulfate is rapid and occurs prior to the WWTP process.

(2) Treatment – Similar to the opportunities for binding that exist in the waste stream traveling to the WWTP, yet greater opportunities exist at the WWTP itself. Kim et al. (2010) found that silver ion, when bound to sulfur compounds in sludge or other available organic ligands, is much less toxic than free silver ions. Additionally, any silver that sorbs onto available organic matter is highly likely to be removed via agglomeration and precipitation out of the water column (Stensberg et al., 2011). While the treatment process removes the vast majority of silver<sup>10</sup>, it has been reported that a small amount of silver ion can pass through these systems into the aquatic environment (Benn & Westerhoff, 2008). Kaegi et al. (2011) investigated the behavior of silver nanoparticles in a pilot WWTP fed with municipal wastewater and found that most of the

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<sup>9</sup> According to EPA (2010), the solubility product constant for silver sulfides is on the order of  $10^{-49}$  to  $10^{-50}$ , which is roughly five times less soluble than silver chloride ( $10^{-10}$ ) and over 15 less soluble than silver acetate ( $10^{-3}$ ). Thus, even in the presence of both sulfate and chloride ions, silver ion has a much greater preference for sulfate over any other anion.

<sup>10</sup> A mass balance exercise for silver in a WWTP published by Shafer et al. (1998) found that over 95% of the influent silver is removed from the water column and not found in the effluent. Lytle (1984) reported similar removal from WWTPs processing silver-bearing photochemical waste streams. Kaegi et al. (2011) also performed a mass balance calculation on their pilot WWTP data and determined that only about 5% of the silver left the WWTP in effluent – in this case, this amounted to 7.2 grams of silver out of the influent concentration of roughly 130 micrograms/liter that was fed into the pilot system for over 3 weeks. Blaser et al. (2008) modeled the fate and transport of silver ions in the Rhine River and found that the “fraction of silver removed by filtration and treatment” to be 85-99%.

silver in both the effluent and sludge was present as silver sulfide and that this reaction occurs rapidly (< 2 hours) under anaerobic conditions. The authors were keen to point out that the rapid transformation time is “considerably shorter than the average hydraulic retention time in the nonaerated tank of the pilot plant (~ 7 h),” implying that there is more than enough residence time in anaerobic tanks of full-scale WWTPs for the “near complete transformation” to silver sulfide. The availability of sulfides (and thus overall sulfidation during the treatment process) was determined by production of sulfide in the anaerobic zones of a sewer biofilm, in an experimental sewer trunk spiked with silver nanoparticles, as reported by Kaegi et al. (2013).

Wang et al. (2012) performed a similar study on a number of nanoscale materials (including silver) for a similar duration using processes and retention times more akin to full-scale WWTP. This work confirmed that nanoscale silver will accumulate in biosolids (rather than effluent) as well as demonstrated that nanoscale silver had “negligible effects on ability of the wastewater bacteria to biodegrade organic material, as measured by chemical oxygen demand” (Wang et al, 2012). Schafer et al. (2013) reported that silver is eliminated from WWTPs with an efficiency of 95-99% through the rapid transformation (less than 30 minutes) of insoluble silver sulfide mainly attached to flocs of sludge, even when receiving “worst case” influent concentrations of up to 20 µg/L. Kaegi et al. (2015) reported very low silver concentrations (less than 0.5 µg/L) in the outflow of a WWTP receiving industrial discharge containing silver chloride and silver sulfide nanoparticles, confirming a removal efficiency of greater than 95%. Furthermore, the fraction of silver chloride present in the WWTP influent was almost wholly transformed to silver sulfide in roughly 30 minutes, during sewer transit to the WWTP. There is also recent evidence that the extracellular polymeric substances (EPS), produced by microbes that reside in biofilm and activated sludge, bind silver ion and act as yet another “sink” for any free silver ion that survives the WWTP process.<sup>11</sup>

(3) Post-Treatment – Similar to the pre-treatment water column, there is ample organic matter and reduced sulfur species in all aquatic environments to act to reduce any remaining silver ion in WWTP effluent and continue to reduce bioavailability and toxicity to aquatic plants (Luoma, 2008). In fact, dissolved sulfides, organic materials and chloride ions will bind up “essentially all the free silver ions in fresh waters (making it unavailable for uptake by organisms) and drive the free silver ions to very low levels” (EPA 2010).

Silver ions can form complexes with available chloride and ammonium ions to form soluble complexes, covalent adducts with proteins bearing thiol groups (e.g. glutathione, cysteine) and insoluble salt precipitates (like silver chloride, silver sulfide).<sup>12</sup> Given that silver is “an extremely particle-reactive metal,” one would expect silver to be “quickly scavenged from the water column, ending up in sediments” in fairly short periods of time (EPA, 2010).

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<sup>11</sup> Geyik, A.G. and F. Cehen (2016). Exposure of activated sludge to nanosilver and silver ion: inhibitory effects and binding to the fractions of Extracellular Polymeric Substances. *Bioresource Tech.* DOI: 10.1016/j.biortech.2016.03.157.

<sup>12</sup> Schafer, B. et al. (2013). State of the art in human risk assessment of silver compounds in consumer products: a conference report on silver and nanosilver held at the BfR in 2012. *Arch. Toxicol.* 87: 2249-2262.



Most recently, a group of federal researchers conducted a 60-day estuarine mesocosm study using both ionic and nanoscale silver (Cleveland et al. 2012) to simulate movement of silver in a “single-level low marsh.” Silver concentrations were then measured via inductively-coupled plasma mass spectroscopy (ICP-MS) in the water column (seawater), sediment, biofilms, hard clams, grass shrimp, mud snails, cordgrass stalks and leaves, and sand. In seawater, silver ion was “completely removed before the first sampling point at 2 h” - which is consistent with the findings of Kaegi et al. (2011) described above.

Among the biota (both plants and animals), only mud snails and grass shrimp showed any accumulation of silver in their tissues from the mesocosm ion treatment. Both of these species feed on detritus and organic matter and, thus, uptake of silver into these species was from consumption of food items to which silver ion had sorbed (and not from the water column, as there was no measurable ion in the water column).

Of particular importance is the fact that, in the ion-treated mesocosm, there was no accumulation of silver into biofilms, sediment or sand. The authors speculate that lack of anaerobic conditions at the water-sediment interface or short residence in the water column prevented transfer of silver ion into sediments, as would be expected given the organic matter and ligand content. Overall, the mesocosm study shows very low potential for accumulation in estuarine biota from the water column and that exposure to aquatic organisms would mainly be expected through consumption of silver-bearing food items. In addition, the study confirms the rapid removal of free silver ion from the aquatic environment, thus minimizing the exposure potential to aquatic organisms.

In fact, the EPA agrees with this assertion, according to the justification it provided in granting an exemption from a tolerance for food-contact uses of SDC products. In addition to FIFRA registration, the SDC products have received an exemption from tolerance (74 FR 27447, June 10, 2009). This was granted specifically to silver ions stabilized with citric acid as SDC, and not extended to “any other silver-containing compounds whether they are other silver salts, complexes with inorganic polymers such as zeolites, or metallic silver in any form or dimension including nanoscale.” Beyond reviewing the low mammalian toxicity of SDC, in granting the tolerance exemption, EPA recognized that, “[T]he uses identified as indoor hard surface applications will result in minimal, if any, runoff of silver into the surface water. The use of silver as a food contact surface sanitizer will result in minimal, if any, runoff of silver into the surface water. This use will result in an insignificant contribution to drinking water exposures.” (74 Fed. Reg. at 27451). In asserting that “minimal, if any” runoff will occur as a result of indoor uses of SDC products, EPA agrees that this use pattern and associated label instructions is unlikely to lead to environmental exposures of concern.

### **There is sufficient data from the public literature to satisfy 850.6800**

The intent of 850.6800 is to “indicate suitable non-inhibitory concentrations of test substances to be used in biodegradability tests” where activated sludge from a “sewage works treating predominantly domestic sewage” is preferred (EPA, 1996). The toxicity of silver ion to a number of individual microbes important to WWTP systems has been well characterized as has the effect of silver ion on activated sludge organisms in lab- and full-scale WWTP studies, using both “bulk” and nanoscale silver. Given the greater potential for nanoscale silver to release

silver ions, these studies can reasonably be assumed to be “worst case” studies.

In general, silver ion is known to be toxic to sludge bacteria and other microbes via generation of free radicals and reactive oxygen species, resulting in disruption of intercellular enzymes, cell membranes, nucleic acid synthesis (Das et al., 2012; Matsumara et al., 2003). This toxicity has been found to differ between *in vitro* studies done on isolated bacteria and *in situ* tests done in lab- or full-scale WWTPs, as silver ion is generally bound under *in situ* conditions (due to the presence of sulfur, organic matter and other ligands) and not bioavailable.<sup>13</sup>

Garcia et al. (2012) defined potential inhibitory concentrations for nanoscale silver, with regard to WWTP biogas production, as did Yang et al. (2012), who found that silver nanoparticles at less than 40 mg/L have negligible impact on anaerobic digestion and methanogenic assemblages (due to little or no silver ion release), which was found to be a no effects concentration for biogas and methane production. Das et al. (2012) likewise reported a range of silver nitrate concentrations (which readily donate silver ion in solution) within which bacterial production was found to be inhibited over a 48-hour period, including an average median effect (the amount needed to inhibit activity by 50%) concentration of 15 – 276 µg/L. A “low observed effect concentration” was found to be within 8 – 66 µg/L. The authors concluded that “bacterial production is unlikely to be inhibited at the low concentrations predicted by these models.” A recent publication by Hernandez-Martinez et al. (2018) provides a compilation of previously published IC<sub>50</sub> values reported for silver nanoparticles using various cultures and methods.

Geyik and Çeçen (2016) reported a 63% decrease in oxygen consumption from the presence of silver ion (as silver nitrate) at 3 mg/L in sludge in short-term respiration tests and the authors reported 24-hour IC<sub>50</sub> for silver ion (as silver nitrate) as between 2.3 and 3 mg/L. Çeçen et al. (2010) similarly reported 21-hour IC<sub>50</sub> values for respiration of nitrifying sludge following exposure to silver ion of 0.33-0.42 mg/L (dependent on whether oxygen or carbon dioxide was measured). A subsequent study by the same group (Çeçen et al., 2015) confirmed that biological sludge rapidly and powerfully takes up free silver ion into the solid/biomass phase and inhibition in respirometric assays is apparent right away; however, this inhibitory effect weakens when the carbon to nitrogen (COD/TKN) rises.

Other groups have reported significant inhibitory effects in specific bacterial strains from exposure to silver nanoparticles, which at high doses, yields toxicity from both ionic silver and particulate matter. For example, see Battin et al. (2009), Neal (2008), Choi et al. (2008), Sharma et al. (2009), and Zhang et al. (2014). It should be noted, however, that many of these studies use doses of silver nanoparticles in the 20-100 mg/L range, whereas estimates of suspended silver nanoparticles in river water were reported to be two orders of magnitude lower (40-320 ng/L) (Das et al., 2012; Blaser et al., 2008).

Liang et al. (2010) also examined inhibition of nitrifying bacteria in sewage sludge from silver ion and silver nanoparticles at 1 mg/L, where silver ion reduced activity by 13.5%. Yang et al. (2013, 2014) examined the impact of silver ion (as 1 ppm silver nitrate) and silver nanoparticles on ammonia- and nitrite-oxidizing bacteria in activated sludge and found no impact to either microbial community from ion. The authors did not detect silver ion in any microcosms and concluded that

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<sup>13</sup> For example, see review of environmental fate of silver in WWTPs in Reidy et al (2013).

interaction between free silver ion and available inorganic ligands (such as chloride or sulfide) or organic matter was responsible for lack of any effect to the microbial community. By contrast, Gao et al. (2017) reported that concentrations of silver ions (from silver nitrate) of 0.05 mg/L inhibited ammonia oxidation by 53% (and the highest dose of 0.5 mg/L led to 94% inhibition).

Sheng and Liu (2011) reported that isolated bacteria from biofilms were highly vulnerable to silver nanoparticles but original waste water biofilms were tolerant to silver nanoparticles at 200 mg/L (which supports the findings of Kaegi et al., 2011). Similarly, Sudheer-Khan et al. (2011) found that *Aeromonas punctata* isolated from sewage sludge tolerated 200 µg/L of silver nanoparticles and growth kinetics were found to be no different than controls. The authors speculated that part of this tolerance was due to capping of particles by exopolysaccharides (confirmed by XAS) (which confirms resistance of biofilms to silver ions, as found by Cleveland et al., 2012).

By contrast, Choi et al. (2009) found that addition of sulfide to a 1 mg/L dose of silver nanoparticles administered to nitrifying bacteria isolated from a WWTP reduced the toxicity by five-fold (5 X). Similarly, Fabrega et al. (2009) reported that the inhibitory effects produced by administration of 2 mg/L of silver nanoparticles was completely removed by addition of Suwanee River humic acid extracts at 10 mg/L.

Hou et al. (2012) found that chemical oxygen demand (COD) was not significantly different upon exposure to nanoscale silver, and that ammonium removal was initially depressed but quickly recovered (as also seen with non-nanoscale silver and reported by Pavlostathis and Maeng, 1998). They concluded that it was unlikely that citrate-capped silver nanoparticles will cause significant adverse effects on chemical oxygen demand and ammonium removal on activated sludge processes. Wang et al. (2012) found that carboxy-terminated capped silver nanoparticles also had negligible effect on COD.

### **There is sufficient data from the public literature to satisfy 835.1110**

The intent of 835.1110 (ASSI assay) is to provide “[i]nformation on sorption potential ... to assess the possibility for the removal of chemical compounds in biological wastewater systems.” (US EPA, 1998) There is an extensive amount of information on the removal of silver ion by WWTPs, including characterization of both influent and effluent from both “bulk” and nanoscale silver-bearing waste streams. Given the greater potential for nanoscale silver to release silver ions, these studies can reasonably be assumed to be “worst case” studies.

In general, bacteria found in wastewater treatment plants remove metal ions, such as silver, by altering the redox state of the metal ion or through biosorption or bioaccumulation. The settling out of sorbed particulates from the water column and subsequent removal of these particulates via filtration is the main mechanism of physical removal. For more information, see Musee et al. (2011), Gallert and Winter (2005), Lovley and Coates (1997) and Chipasa (2003).

Lytle (1984) reported on the average removal efficiency at six WWTPs, including two that processed photochemical waste (mostly from the use of silver nitrate) and industrial silver (from various uses including insoluble metallic silver). This ranged from 83 (industrial) to 95%

(photochemical) removal and no increase in silver in downstream sediments was reported (reported as less than the level of detection, which was 50 ng/L).

Shafer et al. (1998) examined five privately-owned treatment works and found a greater than 94% removal of silver, independent of silver influent concentration. The authors found that any discharged silver was rapidly dissipated by dilution and incorporation into stream sediments. This very high removal efficiency was confirmed by Adams and Kramer (1999) and again more recently by Kramer et al. (2007).

Pavlostathis and Maeng (1998) found that influent silver levels of 1.85 mg/L (from photoprocessing waste) did not affect WWTP biological activity and “practically all” outbound silver was associated with sludge soils (as measured as 1.84 mg Ag/g mixed liquor suspended solids), as opposed to effluent silver not detected at the limit of detection (0.01 mg/L). The authors subjected both fresh and aerobically digested sludge to TCLP testing and found at resultant silver concentrations were 40 times lower than the regulatory limit of 5 mg Ag/L.

Kaegi et al. (2011) studied the mass balance of silver nanoparticles in a pilot WWTP and reported that 97% of silver was associated with sludge or remained in the WWTP, leaving only 2.5% in effluent.

It bears reiteration that the very high removals of silver consistently found in WWTP studies reflect the fact that silver binds with available sulfur and that there are very high levels of sulfide relative to silver in both WWTP influent and effluent – inorganic sulfides are 200-300 times greater than silver per Adams and Kramer (1999). The authors concluded dissolved silver is strongly complexed by ligands in both influent and effluent waters.

## **Conclusions**

ETI H2O requests a waiver for the Activated Sludge Sorption Isotherm (OCSPP Guideline 835.1110) and Modified Activated Sludge Respiration Inhibition (OCSPP Guideline 850.6800/850.3300) for the following reasons:

1. According to the antimicrobial pesticide data requirements at 40 CFR Part 158 Subpart W, the ASSI test (835.1110) is not required for compounds that are highly volatile (but not hydrophobic), highly reactive, or if the log Kow is less than 3. As estimated by KOWWIN, Version 1.67, the log Kow for silver ion is 0.23, indicating very low potential for solubility in water as well as bioaccumulation (ChemSpider, 2012). SDC is comprised of ionic individual components as SDC is completely water soluble.
2. The labels for all the SDC products specify indoor uses only. Instructions for use include application to indoor hard surfaces via a non-aerosol-generating-method and do not require wiping the surface or a potable water rinse. Thus, there is no viable mobility pathway for residual silver to travel into the environment, and the small quantities that do will be mainly transformed to less soluble and toxic forms before, during and after the WWTP process.

3. EPA has previously agreed with this assertion in granting SDC products (in a manner that is separate from silver salts and silver zeolites) a tolerance exemption for use on food-contact surfaces. In this approval, EPA recognized that “[t]he uses identified as indoor hard surface applications will result in minimal, if any, runoff of silver into the surface water. The use of silver as a food contact surface sanitizer will result in minimal, if any, runoff of silver into the surface water. This use will result in an insignificant contribution to drinking water exposures.” (74 Fed. Reg. at 27451).

4. The ASRI assay (850.6800/850.3300) seeks to determine non-inhibitory concentrations of silver ions to microbial organisms present in WWTPs. There is ample evidence from the literature regarding the effects of silver ions on activated sludge organisms and additional information from the SDC-based products contributes informative value neither to the risk assessment process nor to the existing knowledgebase.

5. The ASSI assay (835.1110) seeks to assess the possibility of removal of silver ions by a WWTP. A substantial amount of information exists in the public literature on the removal of silver ion by WWTPs and additional information from the SDC-based products contributes informative value neither to the risk assessment process nor to the existing knowledgebase.

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